TOF separations (e.g., 600-ms pre-scan), an IMS-TOF vector is folded and summed to obtain the total number of ions accumulated in the ion trap during the pre-scan. This information is then used to select the optimum sequence for the following full IMS-TOF scan. In a preferred embodiment a range of sequences from signal averaging mode (1 ion packet release per IMS separation) to 7-bit extended pseudo-random sequence (64 ion packet releases per IMS separation) is loaded in SDRAM and a particular sequence is chosen based upon the total ion signal from the pre-scan.

[0023] FIG. **1** shows the IMS-TOFMS instrument which was employed in dynamic multiplexing experiments. Electrospray-generated ions were accumulated in the ion funnel trap between the entrance and trapping grids, and then released into the IMS drift tube in short pulses. Following IMS separation, ion packets were analyzed with a TOF mass spectrometer.

[0024] FIG. **2**A shows the modulation waveforms applied to the entrance, trapping and exit grids of the ion funnel trap for encoding ion packet introduction into the IMS drift tube. Lower voltage levels in each waveform correspond to the transmission mode while higher voltage levels indicate ion beam blocking. As seen in FIG. **2**A, ions from the continuous source were accumulated in the ion funnel trap for short intervals (lower voltage level at the entrance grid and high voltage level at the trapping and exit grids) and then ejected from the trap in short release pulses (high voltage level at the entrance grid and low voltage level at the trapping and entrance grid). The timing for the repetitive ion accumulation/ejection process was determined by the encoding pseudo-random sequence. As shown in FIG. **2**B, ion accumulation intervals in the ion trap were constant throughout IMS-TOFMS separation. This enabled signal reconstruction without the use of complex weighing functions and made inverse transform procedure robust and applicable to signals of arbitrary complexity. The duration of the accumulation intervals was determined by the encoding sequence, and, therefore, would vary upon its alternation.

[0025] FIG. **3** shows the flow control diagram for the dynamic multiplexed experiments, which can be conducted with any online or off-line condensed-phase separation, including capillary LC, SCX/SAX and CE. Each measurement is preceded by a short pre-scan that is used to determine the total ion signal at a given time during condensed-phase separation. Based on the total ion signal from the pre-scan, the optimum encoding sequence is selected from a set of predetermined sequences using a calibration function. IMS-TOFMS experiment is then performed under the optimized conditions for a desired number of averages, and the system is reset to the pre-scan mode for the following IMS-TOFMS acquisition.

[0026] In one example, the low abundance fractions for the entire IMS separation time scale are used to accumulate ions in the trap, whereas higher bit sequence (and shorter ion accumulation times) are employed for analysis of higher abundance fractions. Referring now to FIG. **4**, FIG. **4** shows reconstructed multiplexed IMS-TOF spectra obtained with two different reverse-phase fractions of a 0.5 mg/mL depleted human blood plasma sample. The spectra were acquired in a fully automated experiment using a set of 25 reverse-phase fractions. FIG. **4**A shows the two-dimensional contour, IMS and summed mass spectra of the IMS-TOFMS signal encoded with a 5-bit pseudo-random sequence. Data were obtained with fraction **14** of the depleted human blood plasma sample. FIG. **4**B displays the reconstructed signal using data in FIG. **4**A. FIG. **4**C shows the two-dimensional contour, IMS and summed mass spectra recorded in the signal averaging mode. This signal was obtained in analysis of fraction **7**. FIG. **4** shows that in correlation with analyte concentrations from a particular fraction, ion accumulation times in the analysis of different fractions was be varied by a factor of 30 that helped address the dynamic range challenge. It should be noted that process allows dynamic multiplexed IMS-TOF experiments on a sample fraction to be performed during collection of the next fraction. Thus fraction collection and data acquisition/analysis overlay. This results in high throughput. For example, a complete 3D analysis of 25 reverse-phase fractions was be conducted in 15 min, with 0.2 min signal averaging in the multiplexed mode per fraction at a duty cycle of >50%. The use of data compression and developed algorithms for the reconstruction of multiplexed IMS-TOF raw vector also enabled on-line signal monitoring and quality control.

[0027] In one application, the detected features reveal statistically different abundance ratios as a result of various biological stimuli (e.g., comparison of blood plasma samples from cancer and healthy patients) will be subjected to tandem MS experiments. Using LC fraction and IMS drift time information, an RF-only ion guide positioned downstream of the IMS drift tube will be dynamically biased using a pseudo-random binary sequence identical to that employed to detect the features of interest and tailored in time to match the drift time of the features of interest. As a result, a multiplexed IMS-MS/MS spectrum corresponding to the interesting species will be detected and then reconstructed with the same algorithm as that used for deciphering parent ion spectra. Fragment correlation to precursor ions and the following identification can be performed by matching the IMS drift time profiles of parent and fragment signals, and then applying commercially available search engines such as XITandem or Mascot that invoke mass accuracy information.

[0028] FIGS. **5** and **6** show reconstructed multiplexed IMS-TOF spectrum of a 50 nM solution of tryptic digest of bovine serum albumin. FIG. **2** shows a signal that was encoded with 5-bit extended pseudo-random sequence. FIG. **3** shows an extracted ion chromatogram of a tryptic peptide of bovine serum albumin (m/z 488.753) corresponding to the IMS-TOF signal in FIG. **2**. As well as an excitation waveform applied to an RF-only multipole to activate collisional dissociation of this peptide. Activation is accomplished by biasing the multipole to lower DC potential that results in an increase in the precursor ion kinetic energy and the fragmentation shown in FIG. **6**.

[0029] The described design for a dynamic multiplexed IMS-TOF platform coupled to an automated LC fraction collection instrument will enable a complete 3D sample analysis in <10 min at an IMS duty cycle of >50 % and a mass accuracy of <5 ppm. This approach will result in automated analysis of >100 technical replicate analyses per day. In addition, the same approach will provide high sensitivity fragmentation data and complete sequence information for biologically regulated species.

[0030] While various preferred embodiments of the invention are shown and described, it is to be distinctly understood that this invention is not limited thereto but may be variously embodied to practice within the scope of the following claims. From the foregoing description, it will be apparent